FACTORS INHIBITING SEED PRODUCTION IN FLORIGRAZE, RHIZOMA PEANUT (*Arachis glabrata* Benth.)

By

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To my parents,

Rev. Donald. C. and Bernice M. Niles,

Baptist missionaries 35 years in Zaire.

You have endowed me with a priceless legacy,

the example of your lives.

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FACTORS INHIBITING SEED PRODUCTION IN FLORIGRAZE, RHIZOMA PEANUT (*Arachis glabrata* Benth.)

By

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Seed production can be a limiting step in breeding programs. The objective of this research was to characterize the causes of poor seed set in 'Florigraze' rhizoma peanut (*Arachis glabrata* Benth.) through laboratory cytological studies and field pollination and growth experiments.

The effect of photoperiod and temperature on Florigraze reproduction was studied at two field locations using automatic plot shaders in cool and warm-season trials. Reproduction was favored by long days (> 12 h) and warm (25 to 28 C) temperatures. Short days (11 h) and cool temperatures (20 to 22 C) reduced flower production, and induced a partitioning shift from shoots to rhizomes. Peg initiation occurred only in the warm season and never exceeded 6% of flowers produced.

Up to 80% pollen germination was obtained in the laboratory in a solution comprising 100 g/kg sucrose, 100 mg/kg boric acid (H_3BO_3), 250 mg/kg $Ca(NO_3)_2 \cdot 4H_2O$, 200 mg/kg $MgSO_4 \cdot 7H_2O$, and 100 mg/kg KNO_3 in deionized water. Pollen became viable in developing buds between 2200 h and 2400 h the night before anthesis and remained viable in the field for several days under cool, dry conditions. Vigorous pollen could be dependably collected from Florigraze flowers throughout the growing season during the first four hours following anthesis.

Cytological studies indicated Florigraze pollen was not germinating on the stigma. No pollen tubes were found within Florigraze styles until pollinations were augmented with a liquid. Of the numerous solutions and techniques investigated, none exceeded the efficacy, which rarely exceeded 50%, of deionized water in inducing pollen tube initiation. Up to 35% of tubes initiated, aborted within the long style (140 to 200 mm) before attaining the ovary. Pollen tube elongation *in vivo* was about 6 mm/h; thus, fertilization occurred 24 to 33 h after pollination. Peg initiation from assisted pollinations was 6 to 13%, of which 50 to 100% aborted after the pegging vines became over-grown by the surrounding forage canopy. Eight seeds were produced in hybridizations with 'Arbrook', another infertile rhizoma peanut cultivar. The principal constraints to seed production in Florigraze were a stigma too dry to induce pollen germination and a high rate of embryo abortion due to competition in dense canopies.

CHAPTER I
INTRODUCTION

The Promise of Florigraze

Florigraze rhizoma peanut (*Arachis glabrata* Benth.) is a persistent, warm-season, perennial forage legume that has generated considerable interest among agronomists and animal scientists in Florida since it was described by Prine (1964) at the University of Florida. It is a productive, high-quality hay and grazing crop which thrives in the well-drained, sandy soils of the southeastern coastal plain.

Florigraze typically produces 10,000 kg/ha/yr dry matter in three cuttings (Breman, 1980). The crude protein of the forage ranges between 15 and 20% and digestibility averages 72% (Beltranena et al., 1981). Voluntary intake by cattle, sheep, horses, hogs, and rabbits is good (French et al., 1987). In a recent study, yearling calves grazing Florigraze had an average daily gain of 0.93 kg, and a gain of 342 kg/ha/yr (Sollenberger et al., In press). Florigraze performs as well as alfalfa (*Medicago sativa* L.) when substituted in feeds.

Once established, Florigraze is drought tolerant, with dry year yields near those of rainy years. Although a relative of common peanut (*Arachis hypogaea* L.), it is virtually immune to leafspot and other peanut diseases and has few insect (Prine et al., 1981) and nematode pests (Baltensperger et al., 1986). Because Florigraze forms a dense sod of rhizomes below the soil surface, it appears to have an excellent ability to extract nutrients from the soil, showing little response to fertilizer amendments (Prine et al., 1981).

The Problem of Florigraze

Although many agronomic and animal research trials have consistently shown the superiority and suitability of Florigraze to the hay and forage production needs of Florida, acreages of established stands in the state remain low. This low proliferation rate highlights the cultivar's main liability: slow establishment (Adjei and Prine 1976; Prine, 1984).

Florigraze rarely produces seed. Propagation is therefore carried out by digging and planting rhizomes. These rhizomes are slow to establish, susceptible to decay and drought, and plants developing from the rhizomes are very poor competitors with weeds. New plantings can require up to three years to attain complete coverage even with intensive weed management. Ultimately, the slow growth rate and risks associated with establishment have resulted in limited spread of the cultivar from experimental plots to farmland.

This limitation might be overcome if seed production could be induced in Florigraze or other rhizoma peanut lines. If abundant amounts of seed could be produced, rapid stand establishment might be feasible because seedlings are generally more vigorous than rhizome shoots. Furthermore, dissemination would be simplified since seeds are more readily stored and shipped than rhizomes. If only a few seeds could be dependably produced, breeding programs could be initiated to screen for more vigorously-establishing varieties or increased seed production.

The Reproductive Biology of Peanut

The reproductive biology of rhizoma peanut is similar to that of common peanut which has been thoroughly studied by Smith (1950, 1954) and others. Figure 1-1 illustrates the basic components of the peanut flower. Flowers arise from nodes on the vine emerging from sheath-like bracts at the petiole insertions. The flower is borne aloft on a long hollow tube called the hypanthium (Smith, 1950). The stigma and anthers are packed tightly within a membranous petal within the flower called the keel. The ovaries are located at the base of the hypanthium near the vine. The style, down which the pollen tube must grow to fertilize the eggs, is a fine, thread-like structure within the otherwise empty hypanthium. After fertilization, the tissue at the base of the ovaries begins to elongate developing into a "peg" that transports the embryo down into the soil (Brennan, 1969).

Rhizoma peanut produces flowers almost continuously from the fourth week after emergence to near the end of the growing season. Common peanut flowers are cleistogamous (Umen, 1933). The anthers dehisce before anthesis packing pollen around the stigma within the keel (Oaks, 1958). Peanut flowers are almost exclusively self-pollinated. Natural out-crossing rates below 1% (Stone et al., 1973) have been reported. It therefore follows that satisfactory reproduction may be obtained with a relatively low number of viable pollen grains.

Oaks (1958) found that less than 20% of peanut pollen was viable. Faucette and Emery (1974) obtained 50% pollen germination but considered 10% satisfactory. Several studies have shown great variability in peanut pollen quality from flower to flower and plant to plant (Faucette and Emery, 1974; Oaks, 1958; Umen, 1933). Pollen viability has been shown to be influenced by many internal and external factors including relative humidity (Lee et al., 1972), temperature (Oaks, 1958), light quality (Chhabra and Malek, 1978), physiological development of the plant (Fortanier, 1957), and time of collection (Oaks, 1958).

After pollination, a pollen tube grows down the "transmittal tissue" within the style. The hypanthium of common peanut typically ranges from 10 to 70 mm (Smith, 1950) in length. Umen (1933) found that average hypanthium length varied with the time of anthesis, the position of the flower on the vine, peanut variety, and season of the year. Pollen tube growth rate down the style has been measured at 7.5 mm per hour (Bolhius, 1958). This rate may be influenced by temperature (Oaks, 1958). Fertilization occurs about 4 h after anthesis in common peanut, usually before noon (Bolhius, 1958; Smith, 1950).

Two or more eggs may exist in the ovary at the base of the hypanthium. After fertilization, the tissue between the ovaries and the vine becomes meristematic forming an elongating peg (Jacobs, 1947). Brennan (1969) proposed that the strong assimilate sink of this meristem is a common cause embryo starvation during this peg elongation phase. He suggested that the single-seeded pods often produced by peanut, were a result of starvation of the most distal embryo.

The efficiency of common peanut reproduction, in terms of seeds produced per flower, may be quite low. Smith (1954) found that while 91% of the flowers succeeded in egg fertilization, only 63.5% of these initiated pegs, and less than 14% developed into mature pods. Although most of the flowers resulted in fertilized ovaries, it appears that pod load was adjusted by embryo abortion (Smith, 1954; Duncan et al., 1978). These embryos were aborted either by a specific control mechanism or by lack of sufficient photosynthates. Duncan et al. (1978) have shown that selection for peanut genotypes with increased assimilate partitioning to developing fruit at the expense of vegetative growth can account for most of the increases in peanut yields that breeders have obtained in the past few decades.

Resource partitioning in Florigraze may be more complex than in common peanut due to its habit of accumulating large carbohydrate reserves in rhizomes. It is difficult to deduce what strategies it uses to partition assimilates between the canopy, the rhizomes, and seeds. Armstrong (1982, 1983) developed a quantitative model for resource partitioning in seed-bearing rhizomatous perennial plants based on a strategy that attempted to maximize both seed production and coverage. His model predicted that one third of the post-maintenance assimilates should be partitioned to seed production. If his assumptions were correct, Florigraze should have considerable seed production potential.

Common peanut has been observed to respond to long day (LD) photoperiods by favoring vegetative growth over reproduction (Emery et al., 1981; Ketring, 1979; Witzenberger et al., 1985; Wynne et al., 1973). Exposure to LD of 14 h or more generally produced plants with greater top growth, increased dry weight, fewer seeds, and lower seed weight. Short days in contrast, appear to favor increased pod numbers, greater pod size, increased yield, and reduced vegetative growth. These photoperiod effects may interact, however, with temperature (Fortanier, 1957; Wynne et al., 1973), genotype (Wynne et al., 1973; Witzenberger et al., 1985) and irradiance (Ketring, 1979; Cox, 1978).

The optimum temperature for reproduction in common peanut has been found to differ markedly from that for peak vegetative growth.

Partitioning may be influenced by temperature (Campbell, 1980; Fortanier, 1957; Jacobs, 1951; Williams et al., 1975). Campbell (1980) obtained maximum vegetative growth but few seed using a diurnal (day/night) temperature regime of 17/20 C. Seed yields were most strongly influenced by night temperature.

Numerous experiments have investigated the effects of synthetic growth regulators on common peanut (Brittain, 1967; Brown and Ethridge, 1974; Gorbet and Whitty, 1973; Ketring, 1977; Ketring and Schubert, 1980; N'Diaye, 1980; Wynne et al., 1979; Witzenberger et al., 1985). While the yield responses of peanut to these substances have been contradictory, some researchers have observed an increase in number of pods, a decrease in average pod weight, and a decrease in vegetative growth; a response similar to that induced by short days.

The Potential of Florigraze

In February 1986, over 100 Florigraze seedlings were discovered by Dr. E. C. French and L. J. Krouse in a field previously dug for rhizomes. Although seed production is well known in rhizoma peanut (Prine, 1964), this was the first report of a number of seedlings found in Florigraze. This finding demonstrated that the cultivar was capable of producing viable seeds and raised the challenge of discovering and duplicating the circumstances that induced their formation. If seeds could be produced dependably, the considerable variability present in wild perennial *Arachis* introductions (Ruttinger and Quesenberry, 1988) could be used to improve this already-promising forage cultivar.

Hypotheses and Objectives

Three hypotheses were tested in concurrent field and laboratory investigations into Florigraze reproduction. The rationale and focus of each study is presented below. An additional objective of this research was to thoroughly characterize rhizoma peanut reproductive processes to aid breeders in the future.

1. **Florigraze flowers are infertile or self incompatible.** This hypothesis was tested by attempting to demonstrate that Florigraze pollen would not germinate in any media and that pollen tubes never occur in the styles.

2. **Florigraze will produce seed under conditions similar to those found in the tropics: 12 h day length, 27 C mean monthly** temperature, **and 80% relative humidity.** This hypothesis was based on the assumption that long days, such as those prevailing in Florida during the summer, induce a partitioning shift promoting vegetative growth at the expense of reproduction. It was assumed that at least 90% of the flowers achieved egg fertilization and that these embryos were aborted during subsequent developmental stages. This hypothesis was tested by attempting to demonstrate peg and pod production in field plots exposed to 11 h photoperiods or treated with the growth regulator daminozide.

3. **The hypanthium is too long, preventing pollen tube arrival at ovaries.** This hypothesis was tested by determining the rate of pollen tube elongation *in vivo* and attempting to demonstrate a high frequency of pollen tube abortion within the style.

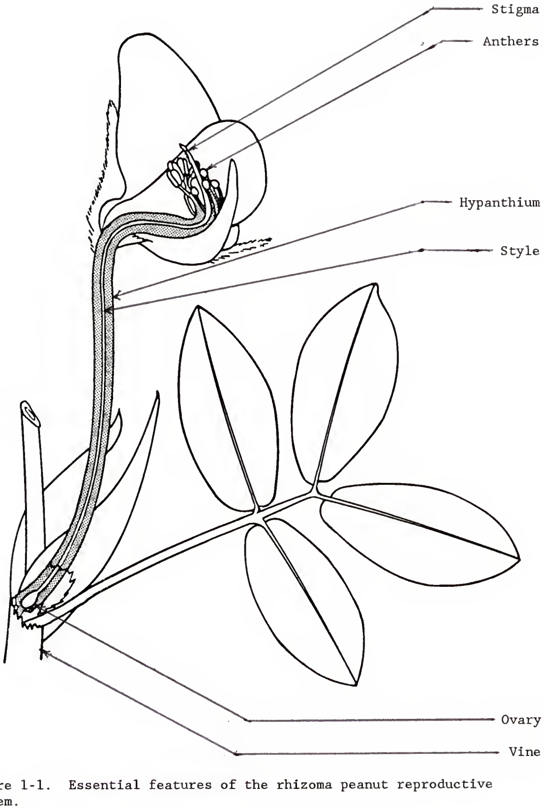

Figure 1-1. Essential features of the rhizoma peanut reproductive system.

CHAPTER II
THE POLLEN BEHAVIOR OF FLORIGRAZE

Introduction

Pollination in common peanut flowers occurs before anthesis (Smith, 1950; 1954). The anthers dehisce packing pollen around the stigma within the keel. Oaks (1958) found this pollen to be less than 20% viable. Faucette and Emery (1974) measured up to 50% pollen germination but considered 10% satisfactory. Variability in peanut pollen quality from flower to flower and plant to plant is widely reported (Faucette and Emery, 1974; Stanley and Linskens, 1974).

The ultimate test of pollen quality is its fertilizing capacity as measured by seed set (Stanley and Linskens, 1974). However when seeds are lacking, pollen sterility must be ruled out as the source of infertility. Pollen germination *in vitro* is one of the most direct and reliable methods of assessing pollen viability (Heslop-Harrison et al, 1984), but these tests are very sensitive to the medium in which they are conducted. The observation that pollen germination was stimulated by the addition of stigmatic fluid extracts to the germination media led to the discovery of several essential nutrients. It is currently accepted that such media must contain a carbohydrate supply, B, Ca, Mg, K, and be adjusted for osmolality and pH (Stanley and Linskens, 1974). Faucette and Emery (1974) reported that a solution containing 100 g/kg sucrose maximized peanut pollen tube growth while 150 g/kg induced maximum pollen germination.

Oaks (1958) investigated the effect of temperature on peanut pollen and pollen tube elongation *in vitro*. Germination only occurred between 18.3 and 35 C while peak tube growth took place at 32.2 C, declining as temperature further increased. He concluded that a large range in diurnal temperature change may be detrimental to the fertilization process particularly when the time interval between the occurrence of temperature extremes is very short.

Faucette and Emery (1974) observed peak peanut pollen viability between 0300 h and 0800 h. Similarly, Oaks (1958) found optimum germination at 0500 h. They recommended that large numbers of flowers be collected over extended periods of time to effectively assess the range of peanut pollen viability.

The overall objective of this investigation was to determine if poor seed production in Florigraze was due to pollen sterility. Specific objectives were (1) to develop an optimum pollen germination solution and (2) to characterize the diurnal and seasonal changes in pollen viability.

Materials and Methods

The same collection and germinating procedures were used in all of the following studies except where indicated. Ten or more flowers were collected per treatment in a sampling transect of a two-year-old Florigraze stand located on the agronomy farm on the University of Florida campus in Gainesville. The flowers were immediately taken to a nearby laboratory where the pollen was removed and bulked. Pollen extraction from mature flowers involved removing the perianth and gently extruding the stigma and anthers through the keel tip. If the anthers had dehisced, the pollen would fall out as a single plug. Additional pollen adhering to the stigma was knocked into the collection dish with a needle. When pollen was extracted from unopened buds, the indehiscent anthers were removed whole with fine tweezers and gently broken open in the dish.

In all but one study, pollen samples were deliberately bulked and mixed before separating them into subsamples. The purpose of combining the pollen was to create a single population of uniform and reduced variance out of which subsamples were taken for testing.

Pollen viability was determined by placing a few drops of germinating solution on a plastic petri dish and inoculating the drops with several hundred grains of pollen. A pollen grain was considered to have germinated when a pollen tube emerged exceeding the grain's diameter. Solutions were prepared by adding varying amounts of sucrose and boric acid (H_3BO_3) to a base solution comprised of 250 mg/kg $Ca(NO_3)_2 \cdot 4H_2O$, 200 mg/kg $MgSO_4 \cdot 7H_2O$, and 100 mg/kg KNO_3 in deionized water. Inoculated drops were incubated 30 min at 35 C by floating the dishes on the surface of a water bath in a tightly closed 30 L styrofoam cooler. Thermal inertia of the bath maintained the desired temperature within 0.1 C over the incubation period. The closed cooler also maintained a high humidity which was essential for good germination.

After incubation, pollen viability was determined by viewing several areas of each drop under a microscope at 40X or 100X magnification and assigning a germination score to the sample ranging from 0 to 5 corresponding to a pretransformed scale of 0, 10, 35, 65, 90, 100% germination (Little and Hills, 1978). Pretransformed scales are designed to adjust for the variance distribution typical of binomial data. These data tend to have variances that are smaller at the two ends of the range (around 0 and 100%) but larger in the middle. A qualitative scoring method was adopted after it was found that the time required to count individual grains introduced substantial time-associated errors. Specifically, the media would evaporate and the pollen deteriorate. As experience was gained in scoring germination, ranges having higher resolutions (more steps) were employed.

The pretransformed scores were used in all analyses. For presentation of results, the means were back-transformed to the appropriate percentage using the equation: percent germination = $(\sin((90°/y)*z))^2$ where y is the number of steps in the scoring range and z is the pretransformed score.

Incubation Temperature and Time

Estimating pollen viability by distinguishing between degrees of pollen staining requires more skill than detecting germinated among non-germinated pollen grains. However, pollen germination tests are inherently more time consuming than differential staining tests because they require an incubation period. In order to reduce this time component, an experiment was conducted to determine the shortest incubation time (requiring higher temperatures) that could be used without risk of introducing temperature-associated degradations in pollen viability. The design was a split-plot with three collection times as main plots, and a 4 x 3 factorial of temperature and time forming subplots. There were three replications. Pollen from three collection times (0000, 0400, 1000 h) was incubated at four temperatures (20, 25, 30, 35 C), for three durations (30, 60, 120 min).

The temperature-time combinations were evaluated immediately after collection. The germinating solution contained sucrose at 80 g/kg and B at 120 mg/kg in the base solution. Three drops of media were placed on each petri dish and three dishes were required for each incubation temperature (one for each incubation period). Four baths were used to allow simultaneous incubation of the pollen samples at the four temperatures. At the end of each incubation period one dish was collected from each bath and the germination scored using a six-step pretransformed scale corresponding to 0, 7, 25, 50, 75, 93, and 100%.

Pollen Germinating Solutions

It is essential to have a solution that will promote maximum germination of the particular pollen sample under investigation when estimating pollen vigor. Six studies were conducted to define the optimum sucrose and B concentration for Florigraze pollen germination.

A preliminary experiment investigated the germination of pollen from three collection times (0600, 0800, 1000 h), in three concentrations of B (50, 100, 200 mg/kg), sucrose (50, 150, 250 g/kg), and Ca ($Ca(NO_3)_2 \cdot 4H_2O$ at 250, 350, 450 mg/kg). The experimental design was a split plot with collection time main plots and solution subplots comprising a 3^3 factorial of B, sucrose, and Ca with three replications.

Subsequent experiments dealt only with sucrose and B in a five by five factorial comprising five levels of each nutrient and replicated five times. Each experiment led to the testing of additional sucrose and B concentrations until a peak response surface was obtained. Over the course of these experiments sucrose concentrations of 30 to 180 g/kg were evaluated in conjunction with B concentrations of 0 to 1000 mg/kg.

The solutions were prepared by diluting stocks of higher initial concentration. Ten milliliters of each solution was autoclaved 15 min at 250 C in glass vials and stored at 5 C. Drops of solution were transferred to plastic petri dish covers using a glass rod or pipette appropriately rinsed between solutions. Each petri dish held all the drops for one complete replication. Drops were arranged in a grid following a pattern inked on the dish bottom. The order of solution drop placement and of scoring was randomized differently for each dish.

Diurnal and Seasonal Variations in Pollen Quality

The preceding studies were conducted concurrently with an investigation into Florigraze pollen behavior throughout the growing season and during the last hours of anther development. Four 24 h studies were conducted that effectively sampled pollen development over a 30 h period. Flowers were collected from 14 h before, to 16 h after, anthesis. Each experiment occurred within a different season, enabling the effects of the prevailing meteorological, edaphic, and physiological conditions to be observed. Analysis of all four experiments would suggest the best season for pollination, if any.

In each 24 h experiment, twenty flowers, ten in anthesis and ten buds, were collected at 2 h intervals. The viability of pollen from these flowers was determined during the interval between collections. In all but one experiment, pollen from the ten flowers or buds was pooled before being divided into four subsamples. In the last experiment, flower to flower variability was estimated by treating each flower as a subsample.

The 24 h studies were conducted 5 Aug 1987, 2 June 1988, 13 July 1988, and 10 Aug 1988. Slightly different germinating solutions were used in each of the studies. Two solutions were used in the first three experiments whereas only one solution, the optimum defined earlier, was used in the last.

Each study was analyzed independently because the number of subsamples and solutions used varied. A combined analysis using only the best solution in each study compared seasonal effects in a model unbalanced only in number of subsamples per season.

Results and Discussion

Incubation Temperature and Time

The temperature x time interaction was significant only for the 0000 and 0400 h collection times (Table 2-1). Germination at 35 C for 30 min did not differ ($P > 0.10$) from the conservative regime of 25 C for 120 min. Peak germination (around 60%) was attained at all incubation temperatures by 120 min (Fig. 2-1). Trends in Fig. 2-1 indicated that shorter incubation periods were possible. Subsequently, incubations as brief as 15 min (data not shown) were found to give equally valid germination scores. In preliminary tests, 40 C was lethal to Florigraze pollen and 38 C produced a decline in viability. Oaks (1958) found no germination in common peanut pollen above 35 C.

Mean pollen germination for the 0000, 0400, and 1000 h collection times were 40.2, 43.7, and 14.3% respectively. Low germination at 1000 h is a typical pollen response to hot, humid weather, as will be seen subsequently.

This preliminary experiment demonstrated that Florigraze pollen was quite viable. It also confirmed the hypothesis that brief incubations at warmer temperatures were as effective as more conservative and time consuming incubation regimes. This hypothesis remained valid over three pollen collection times.

Pollen Germinating Solutions

One collection time (typically 0800 h) was used in the germinating solution studies after the first experiment produced no media-by-collection time interaction. Response to Ca concentration was not significant either. The remaining experiments therefore employed a basic media containing 250 mg/kg $Ca(NO_3)_2 \cdot 4H_2O$, the concentration which induced the highest mean germination in the first experiment. Brewbaker and Kwak (1963) obtained "excellent" pollen germination in a diverse array of plant species using any Ca concentration between 50 and 5000 mg/kg. Faucette and Emery (1974) found 350 mg/kg to be optimum for common peanut.

Because the pollen response to sucrose and B concentration in the initial 3^3 factorial experiment was contradictory, additional experiments were conducted in search of a peak response surface. A factorial experiment comprising five levels of the two constituents was repeated four times using different concentration ranges before the optimum combination was identified.

The optimum sucrose concentration was found in an experiment employing sucrose at 60, 90, 120, 150, 180 g/kg, and B at 60, 100, 140, 180, 220 mg/kg. Pollen response to sucrose concentration was significant and curvilinear (Fig. 2-2). No other effects were significant. The response polynomial equation was $Y = 13.27 + 11.24X - .526\ X^2$ where Y is percent pollen germination and X is percent sucrose in the medium. Solving for the first derivative, peak germination occurred at a sucrose concentration of 106 g/kg. This concurs with

Brewbaker and Kwack (1963) who found 100 g/kg optimum for many species. Faucette and Emery (1974), however, obtained maximum peanut pollen germination at 150 g/kg sucrose.

The pollen response to B was subsequently investigated using sucrose at 100 g/kg and B at the geometrically increasing concentrations of 0, 60, 120, 250, 500, and 1000 mg/kg. As before, the treatments were replicated five times. No differences (Fig. 2-3) were detected between any of the solutions containing B. The control, lacking B, had lower germination. Evidently a small amount of B is essential and high concentrations are not inhibitory. As Brewbaker and Kwack (1963) reported for Ca, any concentration above a certain minimum appears sufficient for good germination.

In summary, differences in Florigraze pollen germination were detected only in response to relatively large changes in sucrose concentration and to the complete absence of B. When present in the germinating medium, no differences ($P > 0.10$) in response to Ca or B concentrations were detectable even when these levels spanned several orders of magnitude.

Diurnal and Seasonal Variations in Pollen Quality

The observed pollen behavior in the four 24 h experiments and the ambient temperature prevailing at each collection time is presented in Fig. 2-4. Experiment x collection time interaction was significant. Each 24 h experiment was therefore evaluated separately. Only the response to the best solution is shown although the solution x collection time interaction was significant in two experiments. The germination behavior was essentially the same among the four 24 h viability studies. However, the June and August 1988 experiments manifested longer periods of elevated pollen viability.

Florigraze pollen suddenly became viable in developing buds around 2400 h the day preceding anthesis. The period of peak viability, wherein germination generally attained 70%, lasted from 2400 h to 1000 h. Pollen vigor declined as ambient temperatures rose. By 2400 h the day of anthesis, viability declined to 0% except when conditions were particularly favorable for survival. Afternoon rains in three of the experiments appeared to speed the decay of pollen in wilting flowers. In June, however, an extended period (26 h) of low-level viability (5 to 10%) was observed most likely due to the lack of rain, lower temperatures, and lower relative humidities.

High afternoon temperatures appeared to depress pollen viability. This depression was not permanent as suggested by the August 1987 and July 1988 studies (Fig. 2-4). The absence of a decline in vigor around 1200 h in the August 1988 study may have been due to the excellent soil water status prevailing at that time. Afternoon showers had occurred every day the previous week.

Flower to flower variability in pollen quality reported by Faucette and Emery (1974) was confirmed in this study. Standard deviations superimposed on the August 1988 study means reflect the variance observed in ten flowers scored individually from each collection period. The standard deviations tend to correlate with the means due to the use of pretransformed scores. The large size of the deviations was a consequence of using scores having only five steps which can only resolve differences of 30% in mid-range. Nevertheless note that the standard deviations vary widely over collection periods. These results confirm the wisdom of pooling pollen samples from many flowers.

Several sources of error may have contributed to the variability observed. Skill required in scoring may have introduced bias between experiments. Variations in pollen density among drops of germinating solution could also bias scores. Drops containing few and widely dispersed grains would likely be scored lower than those containing high pollen densities. Uneven pooling of the pollen may have added to the variability among subsamples, particularly when the pollen was immature and difficult to mix. The activity of thrips (*Frankliniella* spp.) may also have added to this variability.

Thrips were found in flowers at all collection times following anthesis. The intensity of infestation during the August 1988 experiment is shown in Fig. 2-5. Mean number of thrips per flower was 5.35, a level within the range reported by Tappan and Gorbet (1979) who observed up to 8.8 thrips per flower in common peanut. Thrips feed on pollen with the aid of piercing and rasping mouth parts. Hammons and Leuck (1966) state that heavy infestations can result in destruction of the flower keel and its contents. In this study some heavily infested flowers were found to be nearly devoid of pollen by late afternoon. The insects were found in flowers even after the pollen had substantially decomposed. It is possible that some of the afternoon declines in pollen viability were due to thrips damage.

Summary

Up to 80% pollen germination was obtained in a solution comprised of 100 g/kg sucrose, 100 mg/kg boric acid (H_3BO_3), 250 mg/kg $Ca(NO_3)_2 \cdot 4H_2O$, 200 mg/kg $MgSO_4 \cdot 7H_2O$, and 100 mg/kg KNO_3 in deionized water. A reliable estimate of viability can be obtained in 30 min or less when incubated in this media at 35 C.

The pollen developing in buds suddenly matures sufficiently to germinate between 2200 h and 2400 h the night before anthesis. It remains viable for most of the morning thereafter, declining as temperatures rise. Under cool, dry conditions, the pollen remains viable in the field for more than 24 h after anthesis. For breeding purposes, vigorous pollen can be dependably collected from Florigraze flowers for several hours after anthesis, between sunrise and noon.

Table 2-1. Florigraze pollen germination from the five best temperature-by-incubation period combinations at two pollen collection times.

Incubation		Percent germination	
		Collection time[a]	
Temp C	Time Min	0000 h	0400 h
35	60	70.7 a	72.4 a
35	30	66.6 a	66.6 ab
20	120	62.6 a	62.6 ab
25	120	60.1 ab	62.6 ab
35	120	60.1 ab	62.6 ab

[a] Means followed by same letter are not different ($P < 0.05$).

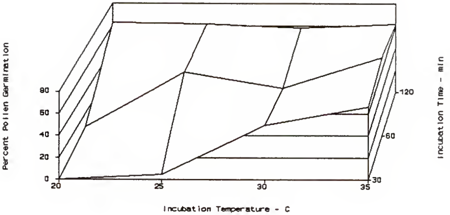

Figure 2-1. Germinating behavior of Florigraze pollen collected at 0000 h when incubated at four temperatures over three different time periods; means of three replications.

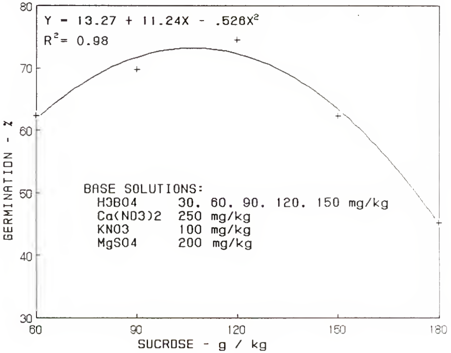

Figure 2-2. Germination response of Florigraze pollen to media sucrose concentration. Means of five replications of a 5^2 factorial of sucrose and B. There was no response to B.

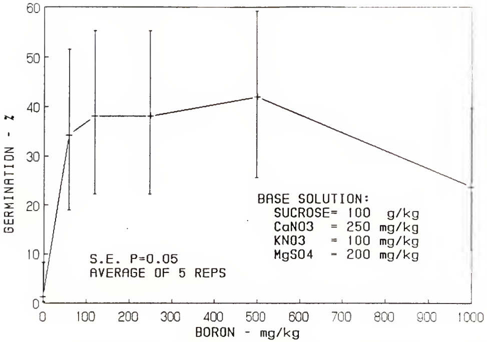

Figure 2-3. Pollen germination response to geometrically increasing concentrations of boron. Average of five replications.

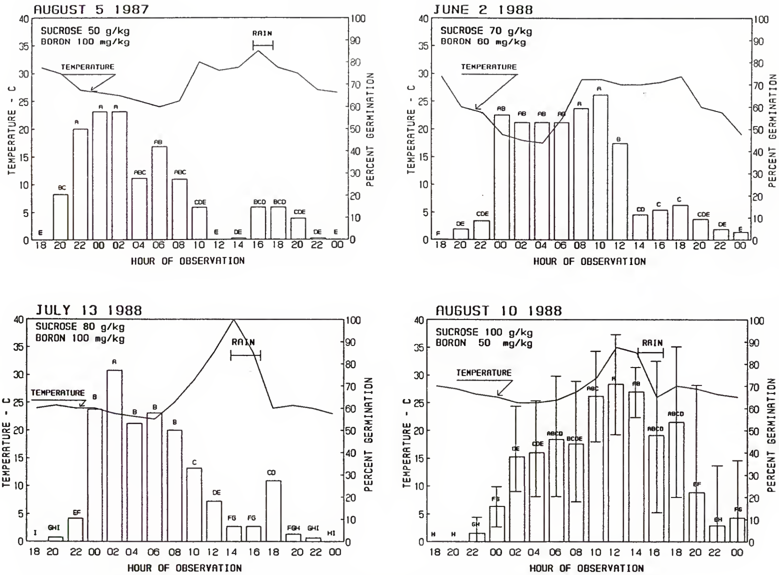

Figure 2-4. Florigraze pollen germination behavior observed in four studies covering 30 h of development from immature bud to wilted flower.

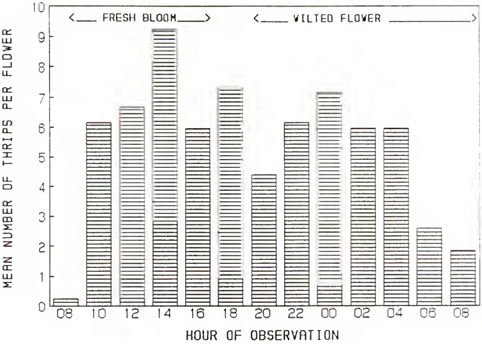

Figure 2-5. Intensity of thrips infestation of Florigraze flowers observed over a 24 h period, average of 25 flowers per collection period.

CHAPTER III
PARTITIONING AND REPRODUCTIVE RESPONSE OF FLORIGRAZE TO PHOTOPERIOD, TEMPERATURE, AND DAMINOZIDE

Introduction

Florigraze response to photoperiod, temperature, and daminozide was investigated to test the hypothesis that seed production is favored by the "tropical" conditions of short days (12 h or less) and 25 C approximate mean daily temperatures. This hypothesis was based on the fact that Florigraze is a tropical plant, an offspring of material collected near the city of Campo Grande in Brazil (Prine, 1964).

Reproduction in Florigraze is very similar to that in common peanut (*Arachis hypogaea* L.) as described by Smith (1950). Flowers arise from nodes in the axil between the petiole and the vine, and are borne aloft on a "calyx tube" or hypanthium. The stamens and pistil are in the flower but the ovary is located at the hypanthium base. Fertilization occurs several hours after anthesis when a pollen tube grows down the style within the hypanthium reaching the ovules. Florigraze flowers have hypanthia much longer than those in common peanut, attaining up to 200 mm in length, a potential constraint to reproduction.

Smith (1954) observed self fertilization in 93% of all common peanut flowers investigated. Pod load was adjusted by embryo abortions during peg and pod development. Typically only 7 to 13% of the flowers resulted in mature pods. This attrition may have been due to competition among embryos (Brennan, 1969) or to deliberate partitioning of assimilates by the plant between vegetative and reproductive sinks. Duncan et al. (1978) showed that recent increases in peanut yields obtained by breeders can be attributed to such partitioning shifts which are apparently under genetic control.

Resource partitioning in rhizoma peanut appears to be more complex in view of its perenniality and habit of accumulating carbohydrate reserves in rhizomes. Postulating what strategies may be used to partition assimilates between the canopy, rhizomes, and seeds is problematic. Armstrong (1982, 1983) proposed a quantitative model of resource partitioning for seed bearing rhizomatous perennial plants based on a strategy that maximizes both seed production and vegetative spread. The model predicted one third of the post-maintenance assimilates would be partitioned to seed. If these assumptions are correct, Florigraze should have considerable seed production potential.

There is abundant published evidence that common peanut responds to long day (LD) photoperiods by favoring vegetative growth over reproduction (Emery et al.,1981; Ketring 1979; Witzenberger et al., 1985; Wynne et al., 1973). Exposure to LD of 14 h or more in general, has resulted in plants with greater top growth, increased dry weight, fewer seeds, and lower seed weight. Short days (SD) have caused reduced vegetative growth and an increased pod-to-flower ratio. However, these photoperiod effects have been observed to interact with temperature (Fortanier, 1957; Wynne et al., 1973), genotype (Wynne et al., 1973; Witzenberger et al., 1985), and irradiance (Cox, 1978; Ketring, 1979).

Partitioning has also been found to be influenced by temperature; the optimum for reproduction differing markedly from that for vegetative growth (Campbell, 1980; Cox, 1979; Jacobs 1951; Fortanier, 1957;

Williams et al., 1975). The optimum day temperature for common peanut growth appears to be near 30 C (Ketring, 1982). The zero growth temperature has been observed to range from 13 to 15 C (Fortanier, 1957; Cox and Martin, 1974; Emery et al., 1969).

Flower production has been shown to respond to photoperiod and temperature. Although common peanut is considered to be indeterminate, peak flowering has been hastened by SD of 6 to 10 h (Ketring, 1979; Emery et al., 1981). Low temperatures have delayed the onset of flowering (Campbell, 1980) and Nicholaides et al. (1969) showed that daily flower production was correlated with the mean temperature prevailing three days earlier. Wood (1967) observed a similar lag and found that a 12 day cool treatment applied soon after flowering onset produced a sustained flowering stimulation to plant maturity. Ketring (1982) concluded that each development phase may have a different temperature optimum and that the sequence of temperatures prevailing in the field when peanuts are normally grown may be far from these optima.

The growth regulating effects of daminozide (succinic acid-2,2-dimethylhydrazide) were first reported by Riddell et al. (1962). Subsequent studies have shown the compound reduces internode length and vegetative spread in peanut, while not affecting leaf area. In some cases yield has increased (Brittain, 1976; Brown et al., 1973) but more often yields have varied, apparently due to differences in genotype, planting date, and seasons (Gorbet and Whitty, 1973; Wynne et al., 1979). N'Diaye (1980) demonstrated that daminozide can induce a partitioning shift from vegetative to reproductive sinks in common peanut when applied to two cultivars having very different partitioning coefficients. The growth regulator (GR) had no effect on leaf area index, growth rate, flower or peg initiation, but raised yield in the low-partitioning genotype to equal that of the high-partitioning one.

Witzenberger et al. (1985) applied daminozide to peanuts under LD and SD photoperiod treatments and demonstrated the GR could partially counter the increased stem growth typically induced by LD. Daminozide also increased vegetative dry weight chiefly by inhibiting leaf drop.

The primary objective of this experiment was to determine if SD of 11 h, or cool temperatures (19 - 22 C), or daminozide, would induce a partitioning shift in Florigraze from vegetative to reproductive sinks. An increase in peg initiation or rhizome biomass production with a concomitant decrease in top growth would be assumed to be evidence of such a partitioning shift.

Materials and Methods

The experimental design was a split-split plot. The main plots comprised a year x location factorial, the subplots comprised warm and cool "seasons" and the sub-subplots comprised two replications of three treatments: an 11 h photoperiod (SD), the growth regulator daminozide (GR), and a control (CTL). The treatments were applied for a (trial) period of six weeks, shown to be the harvest interval producing maximum yield (Beltranena et al., 1981; Brennan, 1980). The cool and warm season subplots were trials conducted as indicated in Table 3-1. The entire experiment comprised four trials conducted within a six-month "window" beginning early in April when temperatures rose sufficiently to promote vigorous Florigraze growth, and ending October 30 when the prevailing photoperiod decreased to 11 h.

The trials were conducted on two fields located on the agronomy farm on the campus of the University of Florida in Gainesville. Two locations were used to generalize treatment effects over two environmental regimes. The fields were nominally 0.03 ha in area. Florigraze had been previously established on them. At the beginning of the experiment, one stand was two years old, the other three; both had attained complete coverage. The younger stand, (location one) was up-the-slope from the older and was bordered on the east by a single row of pine trees which shaded the field during the first morning hours. The soil appeared to be richer at the older field (location two) for it received run-off from several hectares of farmland.

All treatment plots were nominally 0.75 m^2 delineated by pine frames composed of 2.5 x 7.6 cm (1 x 3 in) boards forming a square having internal dimensions of 90 by 85 cm. The frames were anchored in place with stakes.

The following data were collected: pre-trial and post-trial forage biomass, rhizome weight, and pegs per plot. Daily records of flower production and mean hypanthium length were maintained and once a week mean canopy depth was measured. Vine parameters such as leaf area, vine length, nodes per vine, and vine weight were obtained from post-trial samples after the first two trials only. Meteorological data was obtained from the agronomy farm NOAA weather station located 1 km from the experimental locations. Day lengths were computed from sunrise and sunset times published in the Nautical Almanac (1978, 1988) corrected for Gainesville's longitude 82.2° west of GMT.

The rhizome mat below each plot was isolated from its surroundings by cutting the soil deeply along the frame with a spade. At the beginning and end of each trial period, the forage in each plot 2.5 cm above the ground was collected, dried 48 h at 45 C, and weighed. Pre-

and post-trial rhizome biomass was estimated from five soil cores 3.0 cm in diameter 15 cm deep taken in a diagonal transect across each plot. The cores were dried with the forage samples, bulked, sifted to remove sand and gravel, gently winnowed to remove light chaff, and weighed. The resulting mass was adjusted to a 1 m^2 area by multiplying by 283. Five flowering and ten non-flowering vines were extracted from the post-trial forage collections for vine and leaf parameter measurements.

The 11 h photoperiod treatment was applied to the SD plots using automatic shaders developed for this experiment. They were powered by a 12 V battery and incorporated used windshield wiper motors. A digital programmable timer closed the shaders at 1900, opened them at 2200, closed them again at 0430 and opened them at 0800 h thereby maintaining the shaders open for most of the night. The shaders obscured only the last hours before sunset and first hours after sunrise precluding significant temperature elevation within the canopies. The diurnal cycle centered around astronomical noon which occurred at about 1330 h E.D.T. in Gainesville during the summer.

Daminozide (succinic acid, 2,2-dimethylhydrazide) was applied to the GR plots three times on ten day intervals at a rate of 0.95 kg/ha using a wettable powder (85% active ingredients) formulation. The material was applied in a 50 ml volume per plot using a hand sprayer.

The pre-trial rhizome and forage biomass values were used to adjust for plot-to-plot variations in fertility. The post-trial dry weights were multiplied by a constant computed from the pre-trial block (or replication) mean divided by the plot pre-trial value. The rational for this adjustment assumes that a more fertile plot having a pre-trial biomass larger than the block mean would be expected to have a larger post-trial biomass (biasing any treatment effect) and should be reduced proportionally. The adjusted values should represent treatment effects when all plots are of "average" fertility.

Results and Discussion

Photoperiods and Temperatures Encountered

Figures 3-1 and 3-2 present the day length, temperature, and heat units encountered during the four trials. Table 3-1 presents summary statistics for these periods. Note that the temperature minima, maxima, and means for the two cool seasons are remarkably similar but that the prevailing photoperiod differed substantially. Note also that temperatures were decreasing in the 1987 cool season whereas in the 1988 cool season temperatures were increasing.

A complete factorial of photoperiod x temperature treatments was obtained by considering specific CTL and SD treatment combinations over seasons as shown in Table 3-2. Figure 3-3 demonstrates that the effect of warm versus cool temperatures under LD may be discerned by comparing CTL plots alone. Similarly, the SD treatments allow comparisons of the effect of warm and cool temperatures under SD photoperiods.

The field trials did not have equitable water control. Although the fields were irrigated during dry periods in the fall and early spring, the low rainfall and relative humidities of the cool seasons may have introduced drought effects which were confounded with temperature. No reasonable irrigation regime could have equalled the daily afternoon showers and high relative humidities of the late summer in 1987. However, Florigraze is a very drought tolerant crop when fully established (Prine et al., 1981), showing little reduction in yield during dry periods. It is therefore assumed that this deep-rooted forage was less profoundly affected by soil water status than by temperature.

Analysis

Table 3-3 presents the analysis of variance for six characteristics measured in the split-split plot model. Table 3-4 presents the significance of specific mean comparisons for the complete factorial of photoperiod x temperature using means from the model and a single degree of freedom contrast. The GR effect is also compared over several photoperiod x temperature combinations. Figures 3-4 and 3-5 present these season x treatment means.

Location and Year Effects

The yield at location two generally exceeded that at location one in magnitude but there were no differences (P > 0.10) due to location in any of the six response variables. Similarly no differences (P > 0.10) were observed in the response variables due to years. Year x season and year x treatment interactions were found for flowering and hypanthium length (Table 3-3). As will be shown later, these interactions reflect differences experienced by the crop, in the timing of the minimum temperatures during the two cool seasons.

Assimilate Partitioning

The SD treatment reduced forage yield, canopy depth, and vine length as compared to the CTL during the warm season but did not reduce leaf area or number of nodes (leaves) per vine (Fig. 3-4a,b and Table 3-5). Fortanier (1957) found similarly that photoperiod had no effect on common peanut leaf area or leaf number. Ketring (1979), however, obtained a reduced leaf area and leaf number in common peanut under a 12 h photoperiod when compared to a 16 h photoperiod.

The forage yield reduction appeared to be primarily due to the contribution of the internodes, an inference supported by the nearly identical trends in the forage biomass and canopy depth warm season means. It may be assumed that since the leaf areas did not differ, neither did the assimilation rate, and that SD shifted partitioning away from vegetative growth. Had that been the case a significant increase in rhizome biomass or seed production should have occurred in the SD plots. As can be seen in Fig. 3-4c, the rhizome biomass levels were not different ($P > 0.10$) but they did show trends of increasing under SD. The limitations inherent in sampling for rhizome biomass changes plus the large existing rhizome mass present (much of it formed prior to treatment), prevented obtaining significant differences. When the rhizome:forage biomass ratios were considered, however, differences ($P < 0.05$) were obtained suggesting that a partition shift to the rhizome storage sinks did occur in SD. Note, however, in Table 3-6 that peg production was severely reduced in the SD treatment.

Cool season temperatures reduced forage yield, vine length, leaf area, and nodes per vine in most treatments as compared to the warm season. Differences among treatments were reduced in the cool season as might be expected except for the rhizome:forage biomass ratio. The increase in this ratio in cool weather was consistent with observations by Fitzmaurice (1983) who found that rhizome biomass accumulated during the winter and decreased somewhat in the early summer.

The GR treatment caused a reduction ($P < 0.05$) only in forage biomass and only during warm weather. As reported by others (Brown et al., 1973; N'Diaye, 1980), it did not reduce leaf area or node number. The GR did not shift partitioning as indicated by the rhizome:forage ratio. In general, the GR treatment had an effect similar to SD but lower in magnitude. Perhaps at higher application rates the GR would effectively substitute for SD under warm LD conditions as hypothesized.

Reproduction

Short days reduced ($P < 0.001$) flowering and peg production but not hypanthium length as compared to the control. The peg-to-flower ratio which ranged from 0.9 to 6.3% was lowest under SD, contrary to the behavior observed in common peanut (Ketring, 1979; Wynne et al., 1973). Florigraze flowers typically arose from axils three nodes removed from the vine apex. Fortanier (1957) observed that flowering and leaf addition are almost inseparable, however in this experiment, the SD treatment uncoupled flower production from leaf node addition.

Low temperatures reduced flower production, hypanthium length, and suspended peg initiation altogether. Flowering began between 18 and 34 days after the pre-harvest and increased steadily to the end of each trial. Figure 3-5 presents mean flower production by year, season, and treatment. The large discrepancy in flowering behavior between the fall and spring cool seasons (Fig. 3-5c) was probably due to the different temperature and photoperiod regimes prevailing during the latter half of the two trials when flowering began (Table 3-1). During the last (flowering) half of the fall trial, all treatments were exposed to temperatures near the zero growth temperature and to photoperiods approaching 11 h. In the spring the photoperiods exceeded 13 h and temperatures were increasing as the trial progressed. The flowering response to these different regimes likely produced the observed year x season and year x season x treatment interactions (Table 3-3).

Hypanthium length was strongly influenced by temperature (Table 3-4) and weakly by photoperiod (Fig. 3-6, Table 3-3). The response to temperature was curvilinear. Others have also observed a temperature effect on hypanthium length (Fortanier, 1957; Jacobs, 1951). Other experimental observations obtained in this study suggested that hypanthium length was inversely proportional to the quantity and intensity of irradiance received by the flower bud during development (data not shown).

The GR reduced flowering ($P < 0.05$) as compared to the CTL in all seasons but this reduction was not as great in the warm seasons. The GR did not reduce peg production or the peg-to-flower ratio as the SD did. Daminozide reduced hypanthium length irrespective of temperature or photoperiod but this reduction was not large.

Summary

Florigraze vegetative and reproductive growth was most vigorous under warm temperatures and long days. Short days and cool temperatures reduced forage biomass accumulation, canopy depth, vine length, flower and peg production, and shifted assimilates to the rhizomes. Daminozide had an effect similar to, but less potent, than short days. Hypanthium length varied proportionally with temperature. The principal effect of the 11 h photoperiod was the reduction of internode length. It did not reduce leaf area, specific leaf weight, or number of nodes per vine. Contrary to the initial hypothesis, the long days characteristic of the warm growing season in Florida are conducive to rather than an impediment to, Florigraze reproduction.

Table 3-1. Statistical summary of weather observed during four six-week field trials.

	Treatment period			
	Cool season		Warm season	
	1987	1988	1987	1988
Duration (days)	42	42	42	42
First day	19 Sept	8 Apr	14 July	23 May
Last day	31 Oct	20 May	25 Aug	4 July
Temperature (C)				
Minimum	4.4	4.4	20.0	11.1
Maximum	33.3	32.7	37.8	36.1
Mean	21.2	20.6	27.8	25.1
Mean, day 0 - 21	22.9	19.8	27.5	23.8
Mean, day 22 - 42	19.6	21.4	28.2	26.4
Total degree days[a]	250.5	223.3	525.5	411.6
Day length (h)				
Minimum	11.0	12.7	13.0	13.8
Maximum	12.2	13.7	13.9	14.1
Mean	11.6	13.3	13.5	14.0
Total light hours	498.4	570.9	580.7	602.7
Total rain (cm)	4.2	7.9	19.0	11.4

[a] Computed as the sum of daily mean temperatures in excess of 15.6 C

Table 3-2. Treatment and seasonal combinations used to investigate a full factorial of temperature and photoperiod effects.

	Photoperiod	
Season	Short day (SD) Mean photoperiod < 12.0 h	Long day (LD) Mean photoperiod > 12.0 h
	Treatment, Trial, Year	
Warm	SD, Summer 1987, 1988	CTL, Summer 1987, 1988
Cool	SD, Fall 1987, Spring 1988	CTL, Spring 1988

Table 3-3. Model and F test significance from ANOVA for five forage characteristics evaluated.

Source	df	Yield[a] g/m^2	Depth cm	Flower ea	Hypanthium mm	Rhizome g/m^2	Ratio g/g
1 Year (yr)	1	NS	NS	NS	NS	NS	NS
2 Location (loc)	1	NS	NS	NS	NS	NS	NS
3 Main plot error	1						
4 Season (sesn)	1	0.10	0.06	**	***	NS	0.05
5 Year*season	1	NS	NS	***	**	NS	NS
6 Subplot error	2						
7 Treatment (trt)	2	***	**	***	***	NS	0.05
8 Year*trt	2	NS	NS	***	***	NS	NS
9 Season*trt	2	**	*	***	NS	NS	NS
10 Year*sesn*trt	2	NS	NS	***	NS	NS	NS
11 Loc*trt(yr*sesn)	8	NS	NS	**	NS	NS	NS
12 Rep(yr*sesn*loc)	8	NS	NS	**	NS	NS	NS
13 Subsubplot error	16						
14 CV		18.5	25.1	23.0	6.5	20.0	42.2

*,**,***Significant at 0.05, 0.01, 0.001 probability levels, respectively. NS = not significant at the 0.10 probability level.

[a] Yield = Above ground forage dry weight, Depth = Canopy depth, Flower = total flowers produced per treatment period, Hypanthium = Mean hypanthium length per treatment period, Rhizome = below ground rhizome dry weight, Ratio = rhizome:forage biomass ratio.

Table 3-4. Significance of specific mean comparisons compiled from ANOVA interactions and a single degree of freedom contrast as indicated below.

Source	Yield g/m^2	Depth cm	Flower ea	Hypanthium mm	Rhizome g/m^2	Ratio g/g
Day length (LD & SD) within season[a]						
1 LD vs. SD (warm)[b]	***	***	***	NS	NS	*
2 LD vs. SD (cool)[c]	NS	NS	***	NS	NS	**
Season within day length						
3 warm vs. cool (LD)[d]	***	***	**	***	NS	0.10
4 warm vs. cool (SD)[e]	NS	**	NS	***	NS	*
Growth Regulator (GR) within (day length x season)						
5 GR vs. CTL (warm)[f]	*	NS	*	**	NS	NS
6 GR vs. CTL (cool)[g]	NS	NS	*	NS	NS	NS
7 GR vs. CTL (LD*cool)[h]	NS	NS	**	**	NS	NS
8 GR vs. CTL (SD*cool)[i]	NS	NS	NS	NS	NS	NS

*,**,*** Significant at 0.05, 0.01, 0.001 probability levels, respectively.

[a] LD = long day (>12 h), SD = short day (<12 h), warm = summer trials, cool = Spring 1988 and Fall 1987 trials, GR = growth regulator daminozide, CTL = control.

[b] season*trt interaction: SD vs. CTL (warm)

[c] yr*season*trt interaction: SD vs. CTL (Sp 1988)

[d] contrast: ([CTL Summer 1987 + CTL Summer 1988] - 2*CTL Spring 1988)

[e] season*trt interaction: SD cool vs. SD warm

[f] season*trt interaction: GR warm vs. CTL warm

[g] season*trt interaction: GR cool vs. CTL cool

[h] yr*season*trt interaction: GR vs. CTL (Spring 1988)

[i] yr*season*trt interaction: GR vs. CTL (Fall 1987)

Table 3-5. Vine characteristics compared by treatment within summer and fall 1987 trials averaged over two locations and two replications per location. Summer means comprise 40 vines per treatment whereas fall means comprise only 8, therefore combined analysis over seasons was declined.

Parameter average	Warm season[a]			Cool season		
	SD	GR	CTL	SD	GR	CTL
Vine length (mm)	69.1b*	105.9a	112.0a	19.3	16.1	33.1
Nodes per vine	5.1	5.3	5.4	2.5	3.1	3.4
Leaf area (cm^2)	18.1	17.6	20.7	10.1	13.4	15.6
Specific Leaf Weight (mg/cm^2)	6.9 a	6.4 ab	5.4 b	6.4	6.4	6.2

*Means within a row followed by different letters are different ($P < 0.05$). Means within a row not followed by a letter are not different ($P > 0.10$).

[a] SD = short day (<12 h), warm = summer trials, cool = spring 1988 and fall 1987 trials, GR = growth regulator daminozide, CTL = control.

Table 3-6. Peg production by treatment and season averaged over two locations with two replications per location.

Treatment	Treatment period			
	Cool season		Warm season	
	1987	1988	1987	1988
	-----average pegs per treatment-----			
Short day	0.0	0.0	2.0b[*]	0.25
Growth regulator	0.0	0.0	33.5a	0.25
Control	0.0	0.0	36.5a	0.5
Season means	0.0	0.0	23.8	0.33

[*] Means within a column followed by different letters are different ($P < 0.05$). Means within a column not followed by a letter are not different ($P > 0.10$).

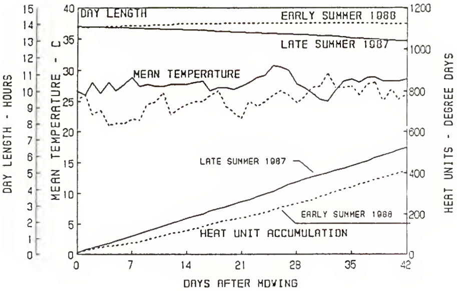

Figure 3-1. Photoperiod, mean daily temperature, and heat unit accumulation (based on the sum of daily mean temperatures in excess of 15.6 C) observed during the 1987 and 1988 summer trials.

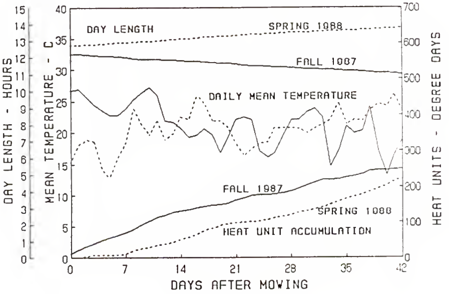

Figure 3-2. Photoperiod, mean daily temperature, and heat unit accumulation (based on the sum of daily mean temperatures in excess of 15.6 C) observed during the fall 1987 and spring 1988 trials.

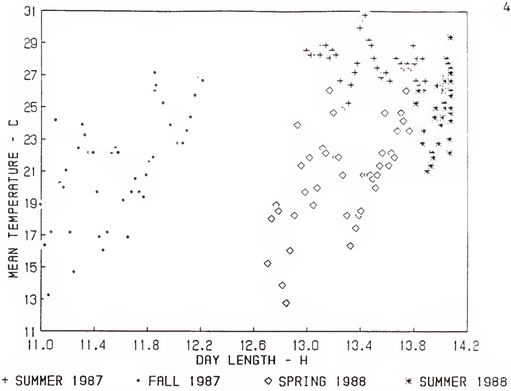

Figure 3-3. The range of photoperiod and temperature combinations obtained during the four field trials conducted over two years.

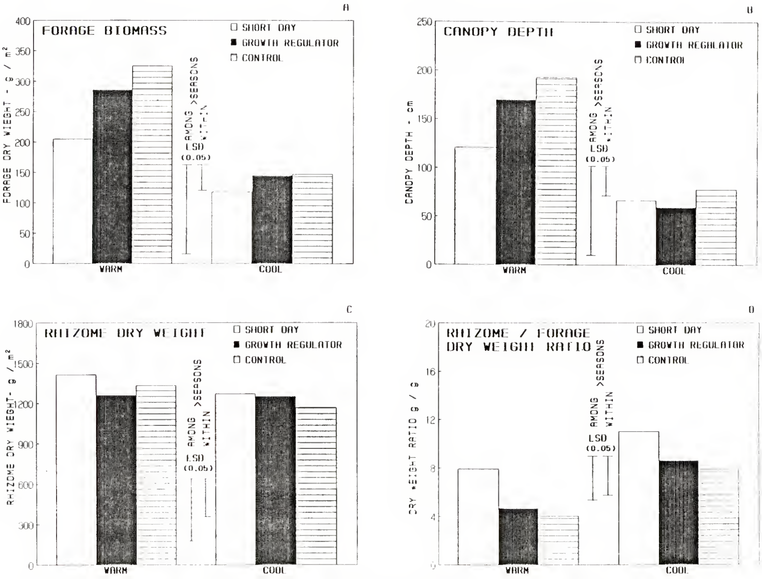

Figure 3-4. Temperature*treatment means of response variables associated with assimilate partitioning.

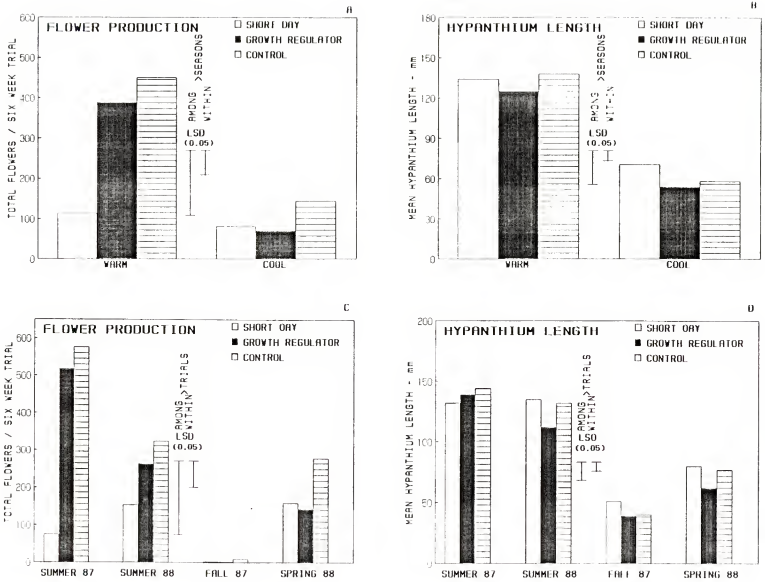

Figure 3-5. Temperature*treatment and year*temperature*treatment means of response variables associated with reproduction.

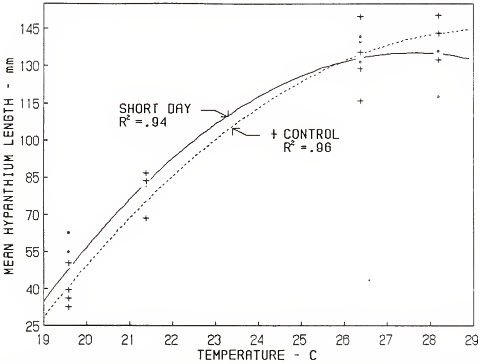

Figure 3-6. Hypanthium length response to temperature and photoperiod. Mean block hypanthium length vs. mean temperature during the flowering period of four trials.

CHAPTER IV
FACTORS INHIBITING SEED PRODUCTION IN FLORIGRAZE

Introduction

In preliminary studies, Florigraze pollen was found to be viable. Peak viability occurred between midnight and anthesis the following morning (Chapter II). Similarly, the hypothesis that seed production was induced by short days or cool temperatures was rejected as a result of trials involving field plots at two locations (Chapter III). Numerous pegs were found in those plots, however, in quantities never previously observed. That research led to this study which attempted to explain that unusual pegging phenomenon and to identify methods of inducing seed production when desired.

One hypothesis investigated proposed that an insect vector was necessary to trip or cross pollinate the flowers. Since the germplasm was collected in South America (Prine, 1964), it was thought that a requisite vector was not imported with the material and that the pegs produced in the earlier field study were induced by the manipulations associated with counting flowers and measuring the hypanthia. The flowers of common peanut (*Arachis hypogaea* L.), are self-pollinated. The natural out crossing rate has been measured by Stone et al. (1973) to be in the range of 0.09 to 0.3%. Since seeds had been lacking, it was not known if Florigraze was self-compatible.

An alternative hypothesis proposed that the excessively long Florigraze hypanthium precluded fertilization. The hypanthia of common peanut typically range from 50 to 70 mm in length (Smith, 1950; Bolhius, 1958) whereas those of Florigraze may exceed 200 mm in length. Since the pollen tube must traverse the style to attain the ovaries at the hypanthium base, it was conceivable that the length of Florigraze styles surpassed the capacity of the pollen tubes to elongate.

A final hypothesis suggested that seed production was inhibited by the strong rhizome and vegetative sinks in rhizoma peanut, causing embryo abortion. According to Smith (1954), 93 of 100 common peanut flowers result in fertilized ovaries, of which only 64 produce elongating pegs, 22 commence pod enlargement, and only 11 produce full seeds. This characteristic of over-setting and then aborting fruit to adjust sink load is well established in common peanut (Duncan et al. 1978).

A series of experiments were conducted to test these hypotheses. A flower tripping study was conducted to determine whether pollination was contingent on flower manipulation by an insect or other agent. The rate of pollen tube elongation within Florigraze styles and the frequency of fertilization was determined using fluorescent microscopy. Finally, embryo abortion was investigated by monitoring the fate of several hundred flowers from pollination to full seed.

Materials and Methods

Flower Cytological Studies

Florigraze flowers were compared cytologically with those of 'Sunrunner' (*Arachis hypogaea* L.), a recently-released commercial peanut cultivar. The stigmas were mounted whole on a slide and stained with 5%

aniline blue in acetic acid. Longitudinal and traverse sections were similarly prepared and viewed.

Flower Tripping Study

Flower tripping was studied in a split-plot experiment having three locations as main plots, and two replications of three subplot treatments at each location. The three treatments involved (1) tripping all flowers manually, (2) excluding all potential vectors with screening, and (3) a control. The 50 cm square plots were located in mature Florigraze fields. In the tripping treatment, all flowers were manipulated daily to promote pollen movement onto the stigma tip. The exclusion plots were caged in a 50 cm cube composed of nylon window screen glued to a wire-frame. The flowers in all plots were counted daily at two locations.

The experiment lasted 8 wk and was repeated twice (13 May to 9 July 1988 and 10 July to 5 Sept 1988). At the end of each experimental period the vines were cut off at the ground and carefully examined for pegs. During the second 8 wk experiment, only two treatments (screen and control) were applied when it became evident manual tripping was ineffective. The total number of flowers and pegs produced and the peg-to-flower ratio, expressed as a percent, was compared among treatments, locations, and experiments. It was assumed that if Florigraze flowers required tripping, pegs would occur in abundance in the tripped plots and perhaps in the control plots if an insect vector was involved, but would be totally lacking in the exclusion plots.

Pollination Study

Pollen germination *in vivo* was studied by pollinating flowers in the field using various treatments and looking for pollen tubes under fluorescent microscopy adapted from Martin (1957). Typically, 10 to 20 Florigraze flowers were self-pollinated between 0600 and 1000 h (E.D.T.) and collected about 24 h later. After the petals (wings and corolla) were removed, the keel and its contents (pistil, stigma, and anthers) and about 30 mm of the hypanthium were transferred to screw-capped tubes containing a fixative comprised of 80% formalin, 10% alcohol, and 10% acetic acid. The flowers remained in the fixative about 30 min until transferred to a laboratory where they were rinsed 5 min in tap water, gently dried with paper towels, and softened 30 min in 8 M sodium hydroxide at 70 C. After softening, the flowers were again rinsed in tap water and transferred to a petri dish for dissection. The dish was placed on a black surface and filled with sufficient water to cover the material. The styles were dissected out of the hypanthium and pistil using sharp tweezers, and were mounted on glass slides with minute petroleum jelly "spots." When several styles had been mounted on a slide it was placed in stain for 30 min before viewing at 100 X. The number of styles containing pollen tubes was recorded.

During the summer of 1988 over 1,300 flowers were pollinated and examined for the presence of pollen tubes. A variety of solutions and techniques were investigated for their potential to enhance pollen tube initiation. The solutions used were indole acetic acid (IAA) and gibberellin (GA) at 12.5, 25, 50, and 100 mg/kg, a pollen germinating solution comprised of 100 g/kg sucrose, 100 mg/kg boric acid (H_3BO_3), 250 mg/kg $Ca(NO_3)_2 \cdot 4H_2O$, 200 mg/kg $MgSO_4 \cdot 7H_2O$, and 100 mg/kg KNO_3 in deionized water, a solution comprised of a trace amount (0.01 μL/L) of the "X-77"®[1] surfactant (alkyarylpolyoxyethylene glycols, free fatty acids and isopropanol) in deionized water, and deionized water alone. The solutions were applied to the stigma before pollinating as a drop from a hypodermic needle or by pressing a moistened cotton swab to the stigma. Other treatments included cutting the terminal 0.5 mm of the stigma before pollinating, and shading the canopy with one layer of nylon window screening after pollinating. Although most pollinations were conducted in the morning, test pollinations were made at all hours from 0000 h to 1600 h (E.D.T.). In addition to self-pollinations, hybridizations were made with Arbrook and Sunrunner.

Typically 10 to 20% of the pollinated flowers were not recovered from the field either because they became "lost" in the dense rhizoma peanut canopy or were eaten by insects or destroyed by some other agent. As a result, most of the data from these studies were unbalanced and had to be analyzed using chi-square comparisons rather than using analysis of variance.

Pollen Tube Elongation Study

The rate of pollen tube elongation *in vivo* was studied using fluorescent microscopy. Florigraze flowers were self-pollinated at 0700 h using an appropriate solution and collected after 4, 6, 8, 10, 12, 14, and 24 h of elongation. The flowers, including the entire hypanthium and ovaries, were collected, fixed, dissected, stained, and viewed as described above. All styles were measured before softening and after mounting in order to adjust for shrinkage that occurred during softening. When present, the pollen tubes were measured using the stage micrometer to the nearest 1 mm.

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[1] Mention of a commercial product does not constitute endorsement by the Univ. of Florida or the Florida Agric. Exp. Stn. and does not imply its approval to the exclusion of other suitable products.

Peg and Pod Production Studies

Pollinations for peg and pod production were conducted concurrently with the pollen tube studies. Typically, ten flowers within a 4 m^2 plot in a Florigraze field were pollinated using an appropriate solution. The node from which the flower emerged was tagged with a piece of color-coded telephone wire. A different code was used each day. The date, solution, and technique employed was recorded. Once each week, all the tagged nodes in the plot were checked for pegs. A second Florigraze self plot was delineated at the edge of the field after the initial location became heavily overgrown and all the pegs aborted. In addition, bi-directional hybridizations were made with Arbrook. A separate 4 m^2 area was used for the Florigraze-by-Arbrook crosses. The Arbrook-by-Florigraze crosses were made on a single two-year-old space-planting located in a rhizoma peanut nursery. No analysis was conducted on the results of these crosses due to the lack of seeds produced.

Results and Discussion

Flower Cytological Studies

The Florigraze pistil, stigma, and anthers were virtually identical microscopically to those of Sunrunner. Both stigmas were pubescent and terminated with a "cup-like" arrangement of hairs capable of encompassing several pollen grains. It was evident from studies of the Sunrunner flowers that pollen tubes are only initiated and only enter the stigma, from within this cup at the tip. A knot of 5 to 10 pollen grains could always be found tenaciously bound to the stigma tip of Sunrunner flowers collected after dawn. These grains were evidently attached to the stigma by pollen tubes for they could only be removed by forcibly pulling with tweezers. Fluorescent microscopy confirmed the presence of numerous pollen tubes within these Sunrunner styles. Contrarily, no knot of pollen was ever found on the tip of non-manipulated Florigraze stigmas, nor were pollen tubes found in flowers randomly collected from the field.

On closer examination, the terminal 3 to 5 mm of the stigma of both species was found to be hollow and to contain a minute amount of fluid. This fluid could be expressed as a droplet visible under a low power (40 X) microscope, by rolling a needle gently along the stigma. The fluid always emerged from the stigma tip suggesting the existence of a canal that was open to the outside. The main difference between the two species was that the tip of the stigma of common peanut appeared moist and gelatinous when collected early in the morning, whereas that of Florigraze was always dry.

These observations confirmed that Florigraze pistils were morphologically intact and potentially functional. The inability of Florigraze to develop a knot of germinating pollen on the stigma tip was apparently due either to the failure of the pollen to become placed in the distal "cup" or to the lack of moisture for germination.

Flower Manipulation Study

A low rate of peg production was observed in all plots, irrespective of treatment, ranging from 1.5 to 13.0 per plot (6 to $52/m^2$) per 8 wk trial (Tables 4-1 to 4-3). This range is similar to that observed in the photoperiod field study conducted earlier (Chapter III). The peg-to-flower ratio ranged from 0.6 to 2.9% which contrasts with the 64% peg-to-flower ratio observed by Smith (1954) in common peanut.

Flower manipulation did not increase peg production nor did excluding all potential pollinators reduce it. Evidently, neither out-crossing nor flower manipulation were a major requirement for reproduction. Flower and peg production did differ over locations (Tables 4-2, 4-3). Location 3 consistently exceeded the other two in peg production in both 8 wk studies. The coefficient of variation for flower production, peg production, and peg-to-flower ratio, was 25.2, 51.5, and 60.1% respectively.

These results suggest that Florigraze flowers have a low rate of self-pollination that is not enhanced by external manipulation. The locational differences ($P < 0.05$) imply that pegging may be influenced by site-specific factors such as soil fertility, moisture regime, and stand age.

Pollination Studies

No pollen tubes were found in any untreated Florigraze flowers investigated. The first treatment employed to induce pollen tube formation was removal of the distal 0.5 mm of the stigma and application of pollen to the shortened stigma. It was assumed that an internal block precluded pollen making direct contact with the stigmatic fluid and by removing the terminal "cup" this block would be removed. This hypothesis was rejected when pollen tubes were not initiated except when additional liquid was added, and this initiation rate was lower than in stigmas that were not cut (Table 4-4).

Pollen tubes were observed, however, when pollinations were made with the aid of a liquid. Hormones have been used by ICRISAT (1982) to enhance seed production in *Arachis* sp. crosses. The use of hormones was also proposed by Halward and Stalker (1987), based on work by Larter and Chaubey (1965), to overcome incompatibility mechanisms among wild peanut crosses. A large variety of solutions was therefore assayed to identify the combination that rendered the highest percentage of pollen tubes per pollination.

The first solution tested was the optimum pollen-germinating solution employed in pollen viability studies conducted earlier (Chapter II). A low rate of pollen tube production (Table 4-5) was observed, but this rate was far below the potential (93%) predicted by Smith (1954). Two growth hormones (auxin and gibberellin) were then appraised for their efficacy at four concentrations each. Those concentrations that appeared most promising were used repeatedly over varied conditions to identify the best. Ultimately, it was found that the control, deionized water, gave as good results as any solution.

A trace amount of the surfactant "X-77"® was added to deionized water to speed wetting the stigma. This solution appeared to enhanced pollen tube initiation. However, considerable day-to-day variations (20% to 81%) were observed when flowers from a single 4 m^2 plot were pollinated using the same solution and technique each day (Table 4-6). When deionized water was compared to the surfactant over a five-day period (Table 4-7), no difference was obtained.

No correlation could be found between temperature, rainfall, or soil water status to explain these temporal differences in pollen tube initiation. Several increases in pollen tube initiation were observed after heavy rains suggesting that plant water status was important. It is probable that the correlation studies failed because meteorological effects were confounded with solutions and techniques used.

Differences were evident in the pollen-tube-initiating behavior of flowers from various micro-locations within the research field. Flowers produced where the forage canopy was taller returned a higher percentage of pollen tubes to assisted pollinations than areas where it was shorter. The hypothesis that this higher percentage was due to shading by the foliage was rejected (Table 4-4) when the pollen tube production of flowers shaded under insect-screen frames and adjacent unshaded flowers did not differ ($P > 0.10$) over four days of pollinations.

In about half of all pollinations, the solutions were delivered with the aid of a syringe and hypodermic needle (bore #20). The stigma was threaded up the bore of the needle to insure complete wetting before pollinating. An increase in pollen tube initiation was obtained (Table 4-4) when a cotton swab was used to apply the solutions. Furthermore, it was much easier to lightly touch a thoroughly-soaked swab to a stigma tip than it was to thread a stigma up the bore of a needle.

The best time for pollination was found to be in the morning just after the anthers dehisced (Table 4-4). Pollinations were made as early as 0000 h the morning of anthesis to as late as 1600 h thereafter. No pollen tubes were found in styles pollinated before 0600 h or after 1400 h. Florigraze pollen readily germinated in an appropriate solution as early as 2200 h the night before anthesis (Chapter II). The failure to produce pollen tubes on stigmas before 0800 h suggests that either the stigmas were not receptive, or the pollen was not fully mature even though it would germinate in certain media.

Most of the pollination studies were conducted on Florigraze using self pollen. Pollen tubes were also found when pollen from other *A. glabrata* (Table 4-8) cultivars was used indicating hybridization might be feasible. Pollen tubes were never found in Florigraze styles when pollen from common peanut, a different species, was used. Halward and Stalker (1987) reported that the failure of pollen to germinate on the stigma was a major constraint to interspecific hybridization in *Arachis*. Hull and Carver (1939) made the reverse cross using *A. glabrata* pollen on three *A. hypogaea* cultivars and obtained embryos that aborted before exceeding a length of 1.6 mm (1/16 in).

Although considerable effort was expended in an attempt to find a solution that would induce the 93% pollen tube initiation predicted possible by Smith (1954) in common peanut, the best solutions rarely averaged better than 50% (Tables 4-6 and 4-7). That this value may in fact be more realistic for the conditions of this research, is suggested by the 56% pollen tube occurrence observed in randomly-collected Sunrunner flowers (Table 4-8) which were naturally self-pollinated.

These results support the conclusion that the main constraint to seed production in Florigraze and related rhizoma peanut cultivars is the lack of moisture on the stigma, which precludes pollen germination. The importance of stigmatic fluid to pollen germination is underscored in a model proposed by Ferrari and Wallace (1977), in which imbibition of stigmatic fluid precedes all other germination events; triggering the hydration, activation, and synthesis of pollen proteins.

Pollen Tube Elongation Rate

After a dependable method of initiating pollen tubes was available, the rate of pollen tube elongation *in vivo* within Florigraze styles could be studied. Nevertheless, obtaining an adequate data set remained difficult. Percent pollen tube initiation remained low. In one trial, 36 pollinations resulted in only 12 pollen tubes and these were not equitably distributed among the five collection times. Furthermore, two of the pollen tubes aborted and two others could not be used because they reached the ovary an unknown time before the last collection. In another trial, 50 pollinations produced only six pollen tubes, of which one aborted and three reached the ovary.

There were additional problems in determining pollen tube length. The styles shrank 12 to 17% during the softening step of tissue preparation. An adjustment for this shrinkage was made by measuring the styles before softening and after mounting. To reduce bookkeeping, a single shrinkage coefficient was computed from the average length change of all the styles in one collection period. This coefficient was used to adjust the pollen tube lengths measured for that group (Table 4-9). A further problem was the large amount of stained extraneous material found in the base of most styles. The final point attained by a pollen tube in this region was difficult to ascertain; a dilemma limited to later collections. In spite of these constraints the pollen tube lengths obtained were remarkably consistent, particularly for the earlier collections (Table 4-9).

Four studies involving 201 pollinations produced 84 pollen tubes of which 13 aborted, 11 reached the ovary, and 60 were used to compute the rate of elongation as shown in Fig. 4-1. Although a curvilinear function could also be fit to the data, a more useful linear function was obtained by excluding the last, error-prone, collection. This linear function indicates Florigraze pollen tubes elongate at about 6 mm/h during the first 14 h. This rate is within the range reported by others. Bolhius (1958) observed an elongation rate of 7.5 mm/hr. Smith (1956) found ovaries were attained in common peanut 9 h after anthesis in flowers having hypanthia from 50 to 70 mm long (5.6 to 7.8 mm/h).

This study allows the rejection of the hypothesis that the long hypanthium of Florigraze flowers is a major constraint to reproduction. The 6 mm/h elongation rate predicts that most flowers, which have hypanthia less than 200 mm long, will be fertilized within 34 h. There was a relatively low pollen tube abortion rate (15.5%), that is probably independent of style length. Although only 42% of the flowers pollinated in this study initiated pollen tubes, 13% of these reached the ovaries within 24 h and no reason was seen to doubt that most of the remaining 87%, not destined to be aborted, would have completed the journey also. Overall, however, these results predict that only 35.7% of Florigraze flowers pollinated will result in fertilized ovaries, a value considerably lower than the 93% reported by Smith (1954) for common peanut.

Peg and Pod Initiation

As indicated in Table 4-10, only 16 pegs (6.9%) were produced from 230 assisted Florigraze self-pollinations. These pollinations were made with the same diverse array of solutions and techniques used in the pollen tube initiation studies (Tables 4-4 & 4-5). Deionized water was employed to produced nine of these pegs. All of the pegs eventually aborted.

Florigraze vines are thin, spindly, and fast-growing. In mature stands such as the one used in this study, the forage becomes very thick, attaining a density of up to 3,000 vines per square meter. At this density, each vine grows vertically and competes with adjacent vines for access to the light at the canopy surface. Some pegs were initiated by nodes as high as 20 cm above the ground. Vines supporting pegs were often rapidly shaded out by the surrounding canopy since they were at a disadvantage having to support an additional nutrient sink. For this reason, the second set of self-pollinations (11 July to 5 Aug) was made at the edge of the field where more light was available and competition would be reduced. Although the pegs at this site lasted longer, the vines were again buried by the expanding canopy front, and ultimately all pegs aborted.

In order to avoid this shading problem, the eight pegs produced from 103 Florigraze x Arbrook crosses received special care. Vines having pegs were held close to the ground by wires to enhance peg penetration into the soil and the competing canopy was clipped away at weekly intervals. Despite these precautions all of the pegs aborted after several months of slow growth. It is possible that clipping neighboring vines reduced the carbohydrate supply to the pegs since rhizoma peanut vines are interconnected by subsurface rhizomes. Florigraze appeared to be a poor maternal plant having thin, delicate pegs and slender leaves and vines.

The reverse cross using Arbrook as the maternal parent produced eight seeds from 116 cross pollinations that produced 15 pegs. Competition for light was not a problem because the Arbrook plants were only two years old and space-planted in a nursery with ample room for vine spread on open ground.

It is evident from the foregoing that other factors constrain seed production in addition to reduced pollen germination on the stigma. Peg initiation per pollination ranged from 7.8 to 12.9% in the above study. If, as suggested in the preceding section, 35.7% of the pollinations resulted in fertilized ovaries then only 22 to 36% of those embryos attained the peg initiation stage. It is possible this very high peg abortion rate is a characteristic of dense canopies only, and would be lower under less competitive conditions. Peg and pod abortion is a phenomenon known to occur in common peanut also (Smith, 1954), and is assumed to be due to nutrient scarcity (Brennan, 1969; Duncan et al., 1978). Peg abortion would explain why pegs are rarely found in established Florigraze stands. Although a low rate of natural peg production may occur, these flourish only briefly as the vigorous canopy shades out the peg-supporting vines.

Summary

The principal constraints to seed production in Florigraze appear to be restricted pollen germination on the stigma due to insufficient moisture and a high rate of embryo abortion in dense canopies apparently due to competition. Pegs were initiated in the field at a low rate ranging from 0.6 to 2.9% of flowers produced depending on location-specific determinants. An insect vector does not appear to be required for pollination. Hypanthium length was not a limitation to fertilization in these studies. Pollen tubes elongated within the style at about 6 mm/h. In this study, 35% of the assisted pollinations resulted in fertilized ovaries, 8 to 12% of which developed into pegs, but only 0 to 8% of which attained full seed. Rhizoma peanut seed production and hybridization is feasible if pollinations are made with the aid of deionized water and the maternal plants are spaced to prevent shading of pegged vines.

Table 4-1. Total flowers, pegs, and percent pegs per flower produced in 8 wk in 0.25 m^2 plots. Average of two replications[a].

Location	Treatments Tripped	 Screened	 Control	Average over treatments
	------- Total Flowers/plot 8/wk -------------			
1	636.5	441.0	376.0	484.5 a
2	267.0	151.5	211.0	209.8 b
Ave. over location	451.8	296.3	293.5	347.2
	--------- Total Pegs/plot 8/wk -------------			
1	10.5	6.5	3.0	6.7
2	1.5	4.0	3.0	2.8
Ave. over location	6.0	5.3	3.0	4.8
	----------- % Pegs of Total Flowers ---------			
1	1.6	1.5	0.6	1.6
2	0.5	2.9	1.5	1.2
Ave. over location	1.1	2.2	1.0	1.4

[a] Only flower production means over locations differ at $P < 0.05$.

Table 4-2. Total peg production in 0.25 m^2 plots. Means of two replications per location[a].

Location	Treatments Tripped	 Screened	 Control	Average over treatment
	Total pegs/plot 8/wk			
1	10.5	6.5	3.0	6.7 b
2	1.5	3.0	4.0	2.8 c
3	13.0	10.0	10.5	11.2 a
Ave. over location	8.3	6.8	5.5	6.9

[a] Only location means are different at $P < 0.05$.

Table 4-3. Mean pegs produced per plot in two eight-week trials conducted over three locations with two treatments and two replications per location[a].

Location	Treatments Screened	 Control	Average over treatment
	-- Pegs/plot, mean of 2 trials --		
1	4.8	4.8	4.8 ab
2	2.3	3.0	2.6 b
3	6.8	10.0	8.4 a
Ave. over location	4.6	5.9	5.3

[a]Only location effects are different at $P < 0.05$.

Table 4-4. Comparisons of the value of various techniques of pollinating flowers in generating pollen tubes. Each total comprises three or more days of pollinations using three or more different solutions.

Technique used	Flowers pollinated	Pollen tubes found	P. tubes per pollination (%)	X^2
	Stigma tip cut vs. stigma not cut			
Cut	44	2	04.5%	
Not cut	39	6	15.4%	1.68
	Solution applied via hypodermic needle or cotton swab			
Hypodermic needle	210	43	20.5%	
Cotton swab	445	173	38.9%	21.83^{**}
	Flowering canopy shaded vs. not shaded			
Shaded	40	11	27.5%	
Not shaded	36	12	33.3%	0.1
	Morning vs. afternoon pollinations			
Morning	53	22	41.5%	
Afternoon	53	10	18.8%	5.4^{*}

*,** The ratio difference of pollen tubes initiated per flower pollinated over techniques is different at $P < 0.05$ and 0.01 respectively.

Table 4-5. Comparison of pollen tube initiation response of Florigraze self-pollinations to 11 solutions averaged over several application techniques and all relevant studies.

Solution applied to stigma before pollinating	Flowers pollinated	Pollen tubes found	P. tubes per Pollination (%)	X^2
Sucrose solution	76	10	13.2%	
	Auxin over four concentrations			
IAA 12.5 mg/kg	10	3	30.0%	
IAA 50 mg/kg	9	1	11.1%	
IAA 25 mg/kg	9	3	33.3%	
IAA 100 mg/kg	58	8	13.8%	4.3
	Gibberellin over four concentrations			
GA 12.5 mg/kg	11	3	27.3%	
GA 25 mg/kg	12	3	25.0%	
GA 50 mg/kg	58	13	22.4%	
GA 100 mg/kg	130	48	36.9%	3.5
	Deionized water vs. Surfactant			
Deionized water	305	76	24.9%	
Surfactant	350	140	40.0%	16.1**

**The ratio of pollen tube initiation is different at $P < 0.01$.

Table 4-6. Comparison of the day to day variation in pollen tube initiation response to pollination using a single solution and technique (surfactant applied with cotton swab).

Date of pollination	Flowers pollinated	Pollen tubes found	P. tubes per pollination (%)	X^2
29 July	11	7	63.3%	
2 Aug	14	4	28.6%	
4 Aug	29	15	51.7%	
5 Aug	11	5	45.5%	
8 Aug	85	50	58.8%	
18 Aug	17	12	70.6%	
24 Aug	18	9	50.0%	
23 Aug	20	4	20.0%	
30 Aug	17	8	47.1%	
31 Aug	11	9	81.1%	
Total: all days	233	123	52.8%	20.0*

*The ratios differ over days at $P < 0.05$ by X^2 test of homogeneity.

Table 4-7. Comparison of the pollen tube initiation response of flowers to deionized water, a surfactant, and no solution over five days. Twenty flowers were pollinated per treatment each day, however, only 64 to 83 of the 100 flowers were recovered.

Method	Flowers pollinated	Pollen tubes found	P. tubes per pollination (%)	X^2
	Deionized water vs. surfactant			
Deionized water	71	28	39.5%	
Surfactant	83	42	50.6%	1.5
	Above solutions vs. no solution			
Control	64	0	0.0%	45.0**

**Either solution is better than none at $P < 0.01$.

Table 4-8. Comparison of the pollen tube initiation response of three cultivar selfs and crosses to pollinations using a variety of solutions and techniques[a].

Pollinations	Flowers pollinated	Pollen tubes found	P. tubes per pollination (%)	X^2
Sunrunner self[a]	41	23	56.0%	
Florigraze self	1196	317	26.5%	
Arbrook self	14	7	50.0%	
Florigraze x Arbrook	45	6	13.3%	
Florigraze x Sunrunner	17	0	0.0%	
Florigraze x Arblick	15	3	20.0%	
Total: selfs & crosses	1328	356	26.8%	11.1*

*Differences among the ratios of selfs and crosses are different at $P < 0.05$.

[a] Values for Sunrunner, a cultivar of *A. hypogaea* L., reflect random collections between 0700 and 1000 h (E.D.T.); the flowers were not pollinated manually as all other entries were.

Table 4-9. Results of a pollen tube elongation study. Ninety styles were self-pollinated at 0730 h with a cotton swab moistened with deionized water.

	Collection time (h)			
Evaluations	1400	1800	2200	0800
Hours of elongation	6	10.25	13.5	24
Styles collected	20	20	21	24
Mean style length				
Fresh (mm)	103.9	116.5	120.2	120.2
Mounted (mm)	90.5	97.2	103.6	105.4
Shrinkage (%)	12.7	16.6	13.8	12.3
Pollen tubes found	10	9	10	21
Aborted	0	0	2	3
Reached ovaries	0	0	0	3
Mean length unadj. (mm)	33.7	54	73.2	87.8
SD	4.4	8.3	6.8	24.9
Maximum length (mm)	40	65	80	119

Table 4-10. Peg and pod production from rhizoma peanut selfs and crosses made June to August 1988 using various solutions.

Beginning date	Ending date	Pollinations made	Pegs produced	Pods produced
Florigraze selfs				
6/13	6/27	84	6	0
7/11	8/5	146	10	0
Florigraze x Arbrook				
6/20	6/27	103	8	0
Arbrook x Florigraze				
6/29	8/5	116	15	8

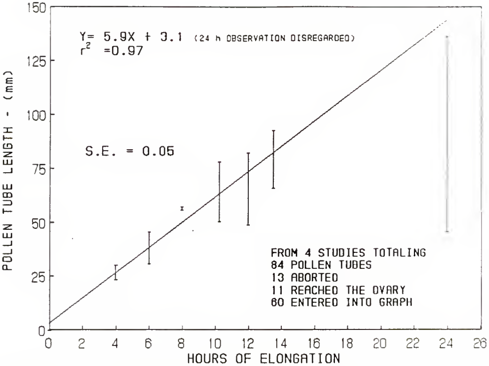

Figure 4-1. Florigraze pollen tube elongation *in vivo*. Bars indicate standard deviations for each collection period.

CHAPTER V
DESIGN AND CONSTRUCTION OF AUTOMATIC SHADERS

Introduction

Growth chambers are commonly used to study photoperiod effects on plants, allowing complete control of an experimental environment. These environments, however, are very synthetic, lacking the rich biotic and abiotic diversity actually existing in the field. Growth chambers are also sophisticated devices requiring diligent care in operation. When maintenance is limited to emergency repairs, as is so often the case, performance gradually deteriorates to a marginally satisfactory level (Downs and Hellmers, 1975; Downs, 1975).

Additionally, few growth chambers produce light of sufficient intensity and spectral quality to adequately simulate that supplied by the sun (Downs et al., 1958). Although relatively efficient, fluorescent lamps emit a stroboscopic light which induces abnormal growth in some plant species (Acock, 1972). Furthermore, fluorescent lighting must be supplemented with incandescent light to supply the far red portion of the spectrum essential for normal plant development (Dunn and Went, 1959).

Finally, growth chamber operation is costly and often beyond the means of limited research budgets. For these reasons, four battery-powered shaders were built to limit day length in the field at a cost estimated to be less than that required to operate a growth chamber for several months.

The shaders were designed with five criteria in mind. First, cost and complexity had to be minimized but not at the expense of dependability. Second, the units had to be built from materials and components that were readily available in local hardware and electronics stores. Third, the systems had to be self-energized, and able to function for at least one week on a fully charged battery. Fourth, the control systems had to be programmable. Finally, the shaders had to completely exclude light from 0.75 m^2 of a forage canopy 30 cm deep.

Materials and Methods

The shading system operated as follows. An open or close cycle began when a timer sent a low current pulse to a relay. The relay then supplied 12 V direct current (DC) to windshield wiper motors which opened or closed the canopy. The motors operated until a position sensor broke the circuit near the end of canopy travel. The DC polarity to the motors was then reversed in preparation for the next cycle which would require motor rotation in the opposite direction.

The schematic in Fig. 5-1 shows the implementation used to fulfill the above design goals. The timer activated a reed relay which momentarily "triggered" a silicon controlled rectifier (SCR). An SCR is an electronic latching relay which, once triggered, remains "on" until current flow is interrupted elsewhere in the circuit. Thereafter, the SCR remains "off" until triggered again. The dual functions of position sensor and reversing switch were combined in a dual-pole-dual-throw (DPDT) switch, which was flipped by a cam follower in the wiper motor. The switch supplied current to the motor in one polarity or the other depending on its position. The cam follower was adjusted to flip the switch, breaking the circuit, turning off the SCR, and reversing the current polarity, when the canopy was 75% fully open or closed. The canopy completed its travel with the aid of gravity and the internal inertia of the spinning motor. A door spring acted as a brake absorbing and storing this kinetic energy for use in the next cycle. Circuit breakers protected the motors and control unit.

Each shader was constructed of 2.5 x 7.6 cm (1 x 3 in) pine boards forming a frame 90 by 85 cm square. The shading canopy consisted of black polyethylene sheeting 6 mm thick attached to metal and plastic tubes 1.25 cm (0.5 in) in diameter. The plastic sheets were fastened by heat-welding short straps of the film around the tubes with the aid of a modified plastic-bag sealer. The tubes rendered a box-like canopy when closed (Fig. 5-2), and a neatly-folded compact package at the shader side when open, much like the convertible top of an automobile. The canopy was opened and closed by the motor rotating the leading tube about pivots at the center of the frame. A 20 cm (8 in) door spring mounted on the frame opposite the motor assisted the beginning, and braked the ending, of canopy travel. The motor was mounted on a plexiglass plate 50 mm thick, drilled and tapped to accept 10-24 machine screws. The plexiglass plate was in turn attached to the frame. A plywood housing protected the motor from the elements. All wood components were treated with a sealing preservative.

The control unit (Fig. 5-3) was sheltered in a wood box 40 x 30 x 23 cm, having a pitched roof and an access door secured with a cabinet lock. The unit housed a timer, 12 V battery, master switch, circuit breakers, and two SCRs (for two shaders). The shaders were connected to the control unit by 3 m of 10 AWG flexible lamp cord. The timer was programed to close the shaders at 1900 h (E.D.T.), open them at 2200 h, close them again at 0430 h, and finally open them at 0800 h imposing an 11 h day length on the forage.

The wiper motors, obtained from late-model Fords[1], were procured from a junk yard. These motors were chosen for their robustness and ability to reverse direction in normal operation. Any other powerful wiper motor would undoubtedly have sufficed. Modifications were made to the wiper drive gear and gearbox cover. The switching mechanism in the cover was removed and a 1.25 cm diameter hole was drilled to accommodate the DPDT toggle switch. The final drive gear was drilled and tapped to accept a threaded peg which served as the cam to flip the toggle switch. A metal fork and mount were made from 1.0 mm thick galvanized sheet metal to serve as a cam follower to throw the switch. The leading canopy tube was bolted to the output arm of the windshield wiper motor with two 10-24 bolts.

The timer was a Radio Shack[1] (p/n 63-891) "Eight Event Program Timer" which runs off an internal 1.5 V "AA" battery. The timer had to be modified slightly to accommodate a 12 V, rather than 110 V, external circuit. The internal relay was replaced with a reed relay and several resistors were replaced. The timer had a minimum "on" time of 1 min whereas the SCRs required only a momentary pulse. The method used to generate this pulse is shown in the schematic. Rather than triggering the SCRs directly, the timer supplied 12 V to the coil of a reed relay through a capacitor. The reed relay closed, triggering the SCRs, only during the brief interval during which the capacitor was charging. The length of this pulse was determined by the value of the capacitor. A bleeder resistor discharged the capacitor in 10 to 20 sec allowing the circuit to be triggered several times in succession without a long discharge delay.

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[1] Mention of a commercial product does not constitute endorsement by the Univ. of Florida or the Florida Agric. Exp. Stn. and does not imply its approval to the exclusion of other products that also may be suitable.

Results and Discussion

Construction of the four shaders and two control units required about 40 man hours once a successful prototype had been developed. No expensive machine tools were necessary and the design was fairly tolerant of unskilled workmanship. Few dimensions required a precision exceeding +/- 2 mm. Holes inadvertently made in the polyethylene canopy during fabrication were easily patched with electrical tape. This tape effected permanent repair withstanding many weeks of exposure to the elements.

Although the shading systems ultimately functioned flawlessly, the first weeks of operation were fraught with the problems typical of a new machine. A liberal dusting of insecticide was required to keep insects from entering the motor covers and gnawing on the wiring. Rain collected in the folds of the open canopies making them too heavy to lift, stalling the motors and blowing the circuit breakers. An additional flap of stiff plastic added to the leading tube successfully kept rain out of the canopy folds. The system of using a cam follower to flip the reversing switch was very sensitive to adjustment. Often a switch would become "hung" midway between positions permanently disconnecting the motor from the control unit. Several weeks of diligent supervision and adjustment were required before all the units operated dependably. Once the above problems were resolved, the shaders functioned perfectly inducing significant short day effects in the forage. The only other maintenance required was periodically mowing back the encroaching forage which threatened to interfere with shader operation.

At the end of each treatment period the shaders were moved to new locations, the canopies were inspected for pinholes and repaired, and the 12 V batteries were recharged. The shaders held up well over the 12 months they were used but the polyethylene canopies became brittle and easily torn toward the end of the last experimental period.

Using mechanical shaders in the field to regulate day length has some limitations. The longest "short day" feasible is restricted to shortest day length prevailing during the treatment period. In this experiment, the shaders could only be used between 15 Apr and 30 Oct, when low temperatures were not limiting and the days exceeded 11 h.

Temperature elevation beneath the closed canopies may also be a problem unless proper precautions are taken. In this experiment, high temperatures never developed because the canopies were closed for no more than 90 min before sunset or after sunrise. During these periods the sun is low on the horizon and radiation is reduced by atmospheric depth.

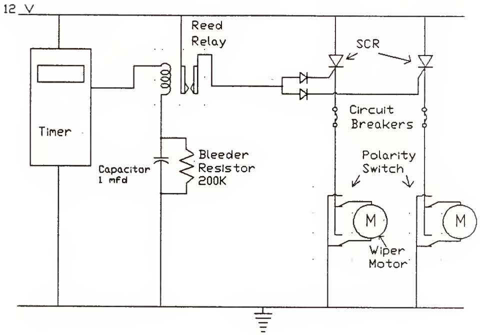

Figure 5-1. Control circuit schematic.

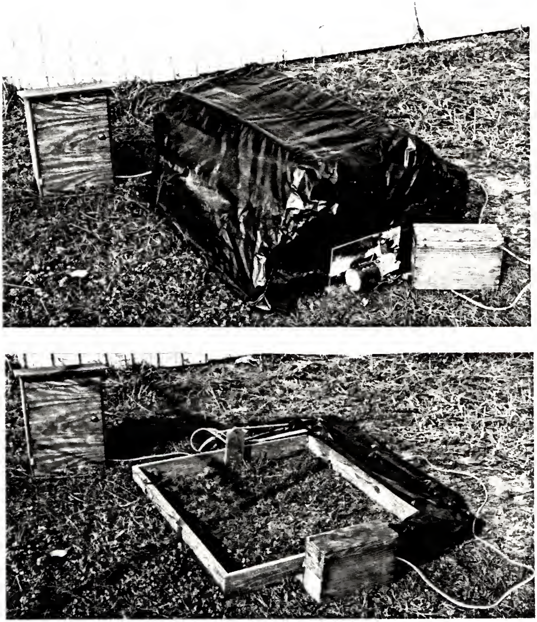

Figure 5-2. Field plot shaders shown with canopy open and closed.

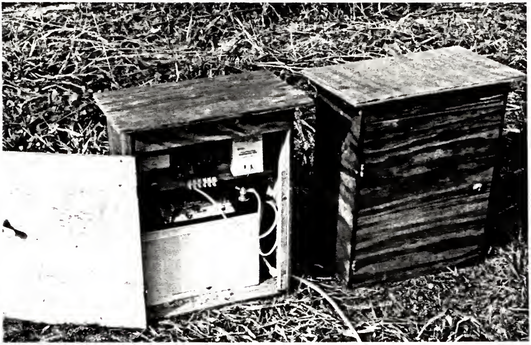

Figure 5-3. Control units enclosing 12 V battery, digital timer, circuit breakers, and SCRs.

CHAPTER VI
SUMMARY AND CONCLUSIONS

Florigraze pollen readily germinated (> 70%) in a solution comprised of 100 g/kg sucrose, 100 mg/kg boric acid (H_3BO_3), 250 mg/kg $Ca(NO_3)_2 \cdot 4H_2O$, 200 mg/kg $MgSO_4 \cdot 7H_2O$, and 100 mg/kg KNO_3 in deionized water. Reliable germination estimates were obtained in 30 min or less using this media at 35 C.

Pollen from developing anthers became viable between 2200 h and 2400 h the night before anthesis in the above solution. Viability was sustained for most of the morning thereafter, declining as temperatures rose. Under cool, dry conditions, Florigraze pollen remained viable in the field for more than 24 h after anthesis. For breeding purposes, vigorous pollen may be dependably collected from Florigraze flowers during the first four hours after anthesis.

Florigraze vegetative and reproductive growth was most vigorous under warm temperatures and long days. Short days and cool temperatures reduced forage biomass accumulation, canopy depth, vine length, flower and peg production, and shifted assimilate partitioning to the rhizomes. Daminozide had an effect similar to, but less potent than short days. Hypanthium length ranged from 10 to 200 mm, decreasing with temperature. The principal effect of an 11 h photoperiod treatment was reduction of internode length. It did not reduce leaf area, specific leaf weight, or number of nodes per vine. The long days characteristic of the warm growing season in Florida appeared to be conducive to reproduction rather than an impediment to reproduction.

The principal constraints to seed production in Florigraze were limited pollen germination on the stigma due to insufficient moisture and a high rate of embryo abortion in dense canopies partially due to competition for light. Pegs were initiated in the field at a low rate ranging from 0.6 to 2.9% of flowers produced depending on location-specific factors. No insect vector appeared to be necessary for pollination. Hypanthium length did not appear to be a limitation to fertilization. Pollen tubes elongated within the style at about 6 mm/h. In this study, 35% of the assisted pollinations produced pollen tubes which attained the ovaries, 8 to 12% developed as pegs, and 0 to 8% produced full seed.

Rhizoma peanut hybridizations and seed production were obtained from controlled pollinations. Pollinations had to be assisted with deionized water and performed within 4 h after anthesis. The maternal plants had to be spaced to prevent shading and abortion of pegged vines. Seed production was favored by conditions which promoted vigorous rhizoma peanut growth such as warm weather and adequate moisture.

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BIOGRAPHICAL SKETCH

Wayne Niles was born May 26, 1954, in Zaire, Africa, the son of Baptist missionaries. He attended high school at the American School of Kinshasa, graduating in 1972. In 1975, he earned a B.S. in mechanical engineering technology at LeTourneau College in Texas. He returned to Zaire to work as a missionary for three years and then for Gulf Oil Corporation the following seven.

After ten years as an engineer, he decided he could make a more meaningful contribution to developing countries with training in agriculture. In 1985 he began work toward an M.S. degree in agronomy with a minor in farming systems at the University of Florida. He completed that degree in 1987 and continued work toward a PhD degree.

Wayne is married to Katherine who is a physician assistant. They expect to spend the remaining years of their lives in rural development work overseas.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Kenneth H. Quesenberry

Kenneth H. Quesenberry, Chair
Professor of Agronomy

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Joseph H. Conrad
Professor of Animal Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Kenneth J. Boote

Kenneth J. Boote
Professor of Agronomy

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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